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From: Murphy, Joseph
Sent: Wednesday, November 13, 2002 4:02 PM
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Please send me the following references:

Wheeler AP, Bernard GR.
Applications of molecular biology and biotechnology: antibody therapy of sepsis.
J Crit Care. 1996 Jun;11(2):77-94. Review.
PMID: 8727029

Verhoef J, Hustinx WM, Frasa H, Hoepelman AI.
Issues in the adjunct therapy of severe sepsis.
J Antimicrob Chemother. 1996 Aug;38(2):167-82. Review.
PMID: 8877531

Thanks a lot...

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APPLIED MOLECULAR BIOLOGY

Applications of Molecular Biology and Biotechnology: Antibody Therapy of Sepsis

Arthur P. Wheeler and Gordon R. Bernard

THE CENTURY-OLD, pioneering studies of von Behring[1] and Kitasato proved that passive immunization (administration of preformed polyclonal antibodies) could attenuate the course of some toxic/infectious human diseases. Traditionally polyclonal preparations, produced in vivo, and composed of mixtures of antibodies directed against numerous sites on one or more antigens, have been used to treat human disease.[2] However, over the last 20 years there has been emerging interest in the therapeutic use of monoclonal products; antibodies produced largely in vitro and all directed at a single structural determinant (epitope) on one antigen.[3] Enthusiasm for monoclonal antibodies was stimulated by the prospect of producing large quantities of pure, homogenous antibody, free of immunodeficiency (and other) viruses that could potentially contaminate animal-derived products. Monoclonal technology was also championed by clinicians dissatisfied with the frequency of allergic or toxic reactions caused by some animal produced antibodies.[4] During these two decades both polyclonal and monoclonal antibodies have been shown to be beneficial and the practice of emergency medicine and critical care commonly uses antibody therapy.

Current examples of polyclonal antibody therapy in clinical use include the following: administration of hyperimmune serum to neutralize tetanus toxin or snake venom[5]; use of hyperimmune immunoglobulin (Ig) G as postexposure prophylaxis for hepatitis B[6]; the use of IgG against RhoD bearing red blood cells to prevent maternal sensitization by an Rh positive fetus[7]; and administration of antihymocyte globulin to treat solid organ transplant rejection. Currently, the only widespread use of applied monoclonal technology for therapeutic purposes is the use of OKT3 (Ortho Biotech, Raritan, NJ) for the modification of lymphocyte populations in the treatment of organ transplant rejection, although other monoclonal antibodies are used as effective diagnostic imaging tools.[8,9] Additional therapeutic clinical applications of antibodies are presented in Table 1, and investigational antibodies are presented in Table 2. Unfortunately, at this time neither monoclonal nor polyclonal antibodies have proven efficacy in the treatment of sepsis.

ANTIBODY BIOLOGY

Excellent reviews of basic antibody biology exist[10,11]; however, a brief review of antibody structure and function is useful before launching into a discussion of therapeutic antibody use in sepsis. Antibodies, regardless of whether they are produced in vivo or in vitro, are proteins produced against antigens recognized as foreign by terminally differentiated B-lymphocytes (plasma cells) and share two basic features: the ability to recognize foreign antigens at specific variable binding sites and the ability to bind to immune effector cells. Human antibodies are composed of basic Y-shaped building blocks constructed from the combination of four peptide chains (two identical heavy chains of ~50-80 kDa each and two identical light chains of ~25 kDa each). The distal portions of the arms of the Y-shaped structure contain variable regions of the antibody specifically designed to recognize and bind antigens. The tail portion of the Y-structure (the Fc fragment) joins the two antigen binding arms, activates the complement, and serves as a recognition site for antibodies to bind to cell surface receptors. Separation of the arms from the tail structure by digestion with papain results in two monovalent antigen binding structures (termed Fab fragments). Similar digestion with pepsin yields a

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Supported by Grant No. HL 43167 and SCOR Grant No. HL 19153 from the National Institutes of Health, National Heart Lung and Blood Institute.

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0883-9441/96/1102-0004$05.00/0

Table 1. Examples of Therapeutic Antibody Use

Product	Origin	Route	Indications
Immune globulin	Pooled human plasma or serum (predominately IgG)	IV	Immunodeficiency syndromes Idiopathic thrombocytopenic purpura Chronic lymphocytic leukemia
Immune globulin	Pooled human plesma or serum (predominately IgG)	IM	Hepatitis A prophylaxis Measles prophylaxis Immunoglobulin deficiency states Vericelle prophylaxis (second line) Rubelle prophylaxis (questionable efficacy)
Cytomagalovirus intravenous globulin	Pooled human plesme with high CMV titer	IV	Cytomegalovirus prophylaxis in renal transplant
Hepatitis B immune globulin	Pooled human serum rich in hepatitis B antibody	IM	Postexposure prophylaxis to hepatitis B
Tetanus immune globulin	Pooled human sarum (Predominately IgG)	IM	Postexposure prophylaxis to tetanus toxin
Varicella-Zoster immune globulin	Pooled humen sarum (IgG, IgA, IgM)	IV	Postexposure prophylaxis to varicella in immunosuppressed host
Rho(D) immune globulin	Pooled human serum	IM	Suppress anti-RhoD antibody formation in RhoD nagative mother
Antilymphocyte immune globulin	Pooled horse serum	IV	Immunosuppression in transplantation Therapy aplastic anemia
Diphtheria anti-toxin	Pooled horse serum	IM or IV	Therapy of diphtheria toxin
Rabies immune globulin	Pooled human serum (Predominately IgG)	IM	Postexposure prophylexis to rabies
Antivenin Crotalid polyvalent	Pooled horse serum	IV	Treatment of pit viper envanometion
Coral snake antivenin	Pooled horse serum	IV	Treatment coral snake envenomation
Black widow Spider antivenin	Pooled horse serum	IV or IM	Treatment Black widow bite
Digibind	Affinity purified Ovine Fab fragments	IV	Treatment of digitalis poisoning
OKT3	Murine monoclonal	IV	Treatment of solid organ rajection

Abbreviations: IV, intravenous; IM, intramuscular.

Table 2. Experimental Therapeutic Antibodies for Sepsis

Product	Origin	Route	Indication
Centoxin HA1A (Malvern, PA)	Murine monoclonal IgM	IV	Humen Gram-negative sepsis (antiendotoxin)
Xomen E5 (XOMA Inc, Berkley, CA)	Murine monoclonal IgM	IV	Human Grem-negative sepsis (antiendotoxin)
CB006 Anti-TNF (Celltech, Slough, United Kingdom)	Murine monoclonal IgG	IV	Human sepsis
CDP571 Anti-TNF- (Celltech, Slough, United Kingdom)	Murine monoclonal IgG	IV	Human sepsis
Bayx 1351 Anti- TNF (Bayen Miles Inc, West Haven, CT)	Murine monoclonal IgG	IV	Human sepsis
CytoTab Anti-TNF (Therapeutic Antibodies Inc, Nashville, TN)	Ovine polyclonal Fab	IV	Human sepsis
MAK 195F Anti-TNF (RASF Knoll Inc, Ludwigshafen, Germany)	Murine monoclonal Fab2 from IgG	IV	Human sepsis

Abbreviation: IV, intravanous.

single V-shaped divalent antigen binding protein (Fab_2 fragment). (Fig 1).

The five classes of human antibodies (IgG, IgM, IgE, IgA, IgD) are determined by the type of heavy chain present and the number of assembled Y subunits. The long-lived IgG is the most abundant immunoglobulin. Present both intravascularly and in extravascular space, IgG serves to protect against bacteria, viruses, and some fungi and parasites. IgA is a secreted immunoglobulin, second greatest in abundance in the human, providing protection against pathogens along mucosal surfaces of the gastrointestinal, genitourinary, and respiratory tracts. Although not usually present in large quantities, IgM provides a vital function in pathogen defense by agglutinating foreign antigens and acting as an efficient activator of the complement system. Critically important because of its rapid generation, IgM serves as a relatively early humoral defense mechanism. Because the molecular weight of IgM approaches 10^6 d, it is

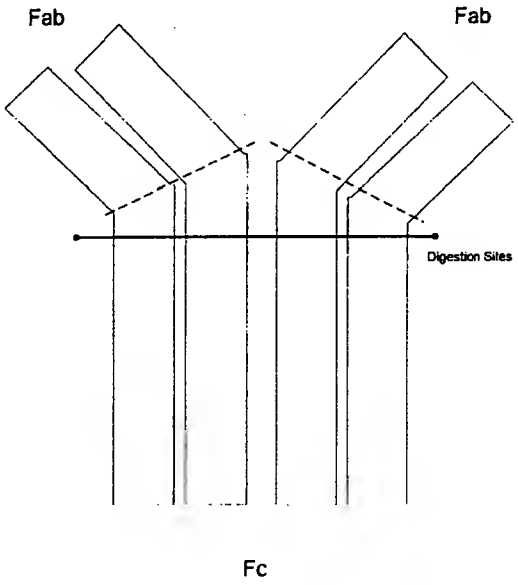

Fig 1. Stylized depiction of the typical Y-shaped basic building block of antibodies composed of two heavy chains and two light chains. In this case a whole IgG structure is shown: Monovalent Fab fragments can be generated from this whole antibody by digestion with pepain (dotted lines) and an divalent Fab2 can be generated by digestion with pepsin at the (solid line).

in large part confined to the intravascular space. IgE, normally found in trace quantities in serum, is predominately a secreted antibody observed lining the respiratory and intestinal tract. Although IgE offers protection from some foreign proteins found in the intestinal tract, it is best known for its ability to activate mast cells in type I (anaphylactic) hypersensitivity reactions.[12] Some nonhuman species produce immunoglobulin classes not found in man, such as the IgY of avian species and IgG(T) found in horses, which are occasionally responsible for the toxicity of an antibody product.[13]

Each class of antibody may be obtained by using either monoclonal or polyclonal production technology, and no profound differences exist between an individual antibody produced using monoclonal or polyclonal technology provided they are of the same class and species of origin and are directed against the same antigenic determinant. Differences between polyclonal antibody preparations and monoclonal antibodies lie predominately in the homogeneity of binding of monoclonal preparations and the heterogeneity of polyclonal preparations. Each type of antibody product has inherent advantages and limitations.

ANTIGEN SELECTION

The first step in producing either a monoclonal or polyclonal antibody for clinical use is appropriate antigen selection. The process of selecting an antigen and optimizing antibody production is complex and well beyond the scope of this article, although excellent reviews of the topic are available.[14] Generally, an ideal antigen is one that generates an exuberant antibody response to a small number of critical epitopes. Usually the best antigens have molecular weights in excess of 10,000 although low molecular weight haptens can be coupled to carrier molecules to increase their antigenicity. The immunization process is also improved in efficiency through use of adjuvants, immunostimulatory compounds administered concomitantly with the immunogen. Proteins and glycoproteins usually represent good antigens; lipids and nucleic acids are less antigenic. When using antibodies for therapeutic purposes, the highest likelihood of success will be occur if the antigen is present in every case of the disease in question; has a constant or near constant structure in vivo; and is present in small quantities in an accessible physiological compartment. For example, it is unlikely that an antibody will benefit a population if only a small fraction of its members have the antigen of interest, as may be the case with endotoxin in septic patients. Similarly, if the antigen is present in a form or physiological compartment, the antibody cannot access therapeutic success. For example, an antibody raised against a nonglycosylated recombinant protein may not be recognized if it only exists in the glycosylated form in patients. Likewise, if a target antigen is only found intracellularly, or only in the extravascular compartment, it is very unlikely that any antibody, or an IgM antibody respectively, could prove therapeutically useful. In the first case this is because the antibody cannot access the intracellular compartment; in the second case it is because IgM antibodies are predominately confined to the intravascular compartment.

Under most circumstances the molar quantity of antibody, or antibody-binding sites, must exceed the number of antigen molecules for

rapid complete antigen neutralization, (defined as prevention of the biological effect of the antigen) to occur.[11] Theoretically, 1 mol of IgM could neutralize 5 mol of antigen because of IgM's pentavalent structure; a mole of divalent IgG could neutralize 2 mol of antigen; a monovalent Fab fragment could neutralize 1 mol of antigen. Unfortunately, antigen-antibody binding is not 100% efficient, so that a given molar quantity of antibody usually binds a lesser quantity of antigen.[11] Furthermore, antigen-antibody binding is reversible. The higher the affinity of an antibody for its antigen the less likely the complex is to dissociate. Dissociation of antigen-antibody complexes can result in the release of free antigen.

Even when present in molar excess, the appropriate test for neutralization of an antigen by antibody is not clear. For example, when testing for endotoxin neutralization, numerous methods are available to detect biological activity. Enzyme-linked immunoabsorbant assays (ELISA) show binding, inhibition of limulus lysate activation, prevention of in vitro cell activation, in vivo pyrogen assays, physiological animal or human studies, or lethal animal models are all possible test beds. The relevance of success or failure in any one of these models is of questionable significance. Because the total number of antigen-binding sites on antibodies must usually equal or exceed the number of antigen molecules for complete neutralization, antigens present in large quantities can be difficult to neutralize. For example, when an antibody is used in an attempt to neutralize a drug overdose or poisoning (eg, digitalis or tricyclic antidepressants) many grams of drug may have been absorbed necessitating use of large doses of antibody. Fortunately in the therapy of sepsis, delivering sufficient antibody is usually not a problem because most target proteins (eg, tumor necrosis factor [TNF], endotoxin) are present in very small quantities.

After formation, large antigen-antibody complexes can be rapidly cleared (within minutes) from the circulation by the mononuclear phagocytes of the liver spleen and lungs. The presence of Fc receptors aids in the reticuloendothelial clearance of these complexes.[15] Smaller antigen-antibody complexes can be excreted in the urine. However, the process of antigen-antibody clearance is poorly understood and several key questions remain unanswered, such as the importance of the complement system in clearing complexes and the reason some animals develop disease from deposition of antigen-antibody complexes.

ANTIBODY PRODUCTION TECHNIQUES

Polyclonal Antibodies

The production of polyclonal antibodies is outlined in several comprehensive works.[11,14] Briefly, polyclonal antibodies are produced by isolating the immunoglobulin fraction of plasma collected from animals spontaneously exposed to, or vaccinated with, an antigen (immunogen). The subsequent humoral response produces antibodies to many different antigen binding sites (epitopes), each derived from a unique plasma cell clone. A polyclonal response represents the immune systems best first guess at which epitopes are important for appropriate disposal (clearance or neutralization) of an antigen. This initial response produces numerous antibodies each of relatively low-binding strength or affinity. The average affinity of all antigen antibody interactions is referred to as avidity. A polyclonal antibody's avidity is often substantially higher than the individual binding strength of any of its components. With repeated immunization and the passage of time, less effective clones become senescent whereas the more effective clones proliferate. During this time, affinity may increase 1000 fold.[16] Eventually, only a few clones producing high-affinity antibodies endure and immunological memory is generated. In this process the immunogen and its method of administration can have a dramatic impact on the quantity and quality of antibody production. The antigen's molecular weight, dose, administration schedule, injection site(s), and adjuvant selection influence the titer and affinity of the resulting antibodies. Adjuvants, immunostimulatory compounds injected simultaneously with antigens, can dramatically boost antibody production; however, their mechanisms of action are poorly understood. Substantial empiricism and art are required for optimal polyclonal antibody production.

Phlebotomy, antibody precipitation, and purification are key steps in production of a biophar-

maceutical grade product from polyclonal antisera. Although a complete discussion of the options for antibody purification are beyond the scope of this article, several basic methods are commonly used to isolate antibodies. These procedures include precipitation with simple salts (ammonium or sodium sulfate) or protein A, hydroxyapatite, or diethylaminoethyl (DEAE)-matrix to yield whole immunoglobulin fractions; DEAE-gel filtration methods to preferentially obtain IgM; or caprylic acid or low-salt protein A combinations to obtain IgG.[17] Further refinement to isolate antibodies with specific binding characteristics can be accomplished in vitro by combining the desired antibody with the antigen of interest or with a second antibody to the desired immunoglobulin using solid-phase affinity purification or immunoprecipitation techniques. When desirable, these isolated antibodies can be enzymatically digested using papain or pepsin respectively to produce Fab or Fab_2 fragments. These antibody fragments have superior tissue penetration, a rapid distribution half-life, and more rapid clearance than intact antibodies.[18]

Precipitation, dialysis, digestion, and immunoaffinity procedures all serve to remove plasma contaminants such as albumin, complement, and clotting proteins, and antibodies directed against compounds other than the target antigen resulting in pure, high-titer, high-affinity antibodies, or antibody fragments from polyclonal antisera.

Monoclonal Antibodies

The initial step in monoclonal antibody production is identical to that for a polyclonal antibody: an animal (sometimes the human animal) is immunized with a target antigen. Hence, the ultimate source of all antibodies, monoclonal or polyclonal, are animal B-cells. After immunization, splenic B-cells are harvested, isolated, and fused with immortalized myeloma cells in a process first described by Kohler et al.[19] For the most part, successful hybridoma formation has been limited to rodent fusion products. Hybridoma production is necessary because nontransformed plasma cells cannot be maintained in long-term culture, and in vitro immunization of B-cells is not feasible. After fusion of thousands to millions of splenocytes and myeloma cells, successfully formed hybridomas are isolated and screened for antibody production. Only a tiny fraction of the fusion products (typically several dozen to a hundred) will produce antibodies that are even candidates to be tested as therapeutic agents. Typically, screening for antibody production is performed using semiautomated ELISA-binding assays. Any clone not producing antibody that binds to the target immunogen is eliminated from consideration. Even when an avid antigen-antibody bond is detected, neutralizing activity is not guaranteed and substantial additional testing is required to confirm biological activity. Once a small number of clones producing antibody with desirable binding characteristics are identified, they are expanded to produce sufficient material to investigate neutralization the antigen of interest in relevant test systems. As one might imagine, the process of isolating and testing monoclonal antibodies can be tedious, time consuming, and expensive.

COMPARISON OF POLYCLONAL AND MONOCLONAL ANTIBODIES

A dogmatic proclamation of the superiority of monoclonal or polyclonal antibodies is impossible because polyclonal products have numerous antigen-binding sites, whereas monoclonal antibodies have a single binding site. In a particular therapeutic setting, the two antibody types could be equally effective or could be superior to the other. For example, in diagnostic immunochemistry the epitope specific nature of monoclonal antibodies usually makes them clear winners. In contrast, using a monoclonal antibody to attempt to simultaneously neutralize multiple complex antigens, such as the treatment of snake envenomation, is likely to fail. The ultimate evaluation of antibodies produced by either monoclonal or polyclonal methods must come through preclinical and clinical studies in which there is a convincing demonstration that the antibody not only binds to its target, but that that binding results in a desirable biological effect. With regard to sepsis this usually implies neutralization of an initiator (eg, endotoxin) or a mediator (eg, TNF, interleukin [IL] 1) of the septic response. The appropriate target for antibody therapy in sepsis is controversial and is discussed later in this manuscript.

Multiple Epitope Binding

Having declared an inability to proclaim a winner in the polyclonal or monoclonal war, several salient points of comparison should be made. Because polyclonal preparations bind numerous epitopes, the thcoretical likelihood the active or critical site of an antigen will be bound is maximized. Conversely, a monoclonal antibody binding to a single epitopc may attach to a critical site or could link to an irrelevant location, the latter situation resulting in an antibody that binds but does not neutralize the biological activity of the antigen. Specifically, multiepitope binding of polyclonals may be critical for effective neutralization of complex antigens or antigcns with a varied stereochemical appearance in vivo compared with that in vitro. An antigen's association with host proteins, or its structural alteration by varied pH or osmotic conditions, can often change epitope geometry and even subtle differences ean prevent antibody-antigen interaction.[20] A practical example of this problem occurs with antibodies to Gram-negative bacterial endotoxins whose outermost oligosaccharide structure can vary depending on growth rate and incubation conditions of the endotoxin-producing bacteria. In rapid growth conditions the oligosaccharide chain may remain incomplete, resulting in altered antigenic appearance.

The potential interactions of several different IgG class antibodies, all of which bind to the antigen of interest, are shown in Fig 2 and 3 using an antigen with a small number of epitopes like TNF-α. Panel A shows the ideal situation in which a monoclonal antibody binds and neutralizes the critical structural determinant, active site A on the toxin. In theory, an antibody to epitope X or Y could be equally effective in stearically blocking this active sitc A. Panel B shows a monoclonal antibody binding to the same antigen with equal affinity at site Z, remote from the active site A. Because the antibody does not directly recognize the active site or any closely adjacent epitope it fails to neutralize thc antigen. In this worst-case scenario, failure to neutralize the active site could result in a circulating antigen-antibody complex with preserved activity and greater longevity in the circulation. (Note the difference between these two antibodies would not likely be detected using only an in vitro binding assay.) Although this example uses a simple antigen, as the number of epitopes increase, it becomes less and less likely that a single randomly generated monoclonal antibody will neutralize the critical active site. Furthermore, a monoclonal antibody is possibly less likely to result in cross-linking, as seen with polyclonal antibodies.

Contrast the situation shown in Fig 2 using a monoclonal antibody with that in Fig 3 using polyclonal antibodies. Administration of polyclo-

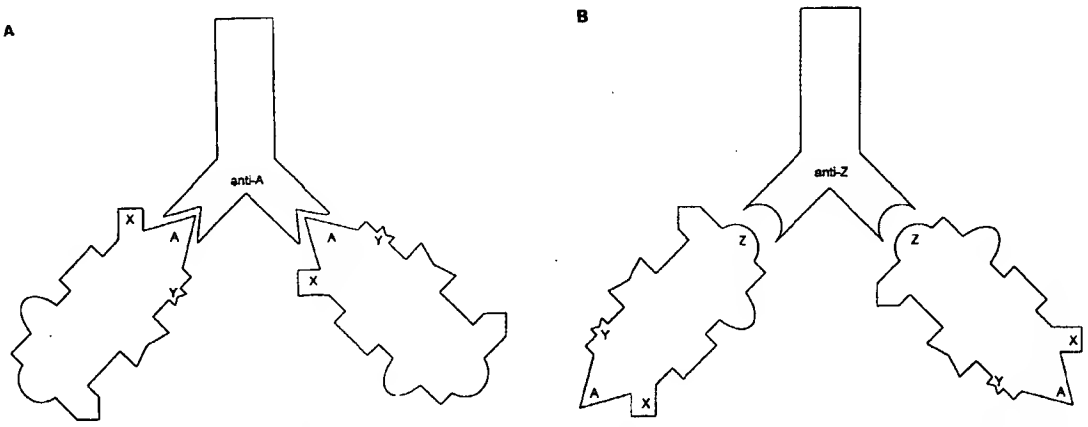

Fig 2. Panal A. The a monocional antibody thet both binds end nautrallzes the critical structural datarminant, activa sita A on the toxin. In theory, an antibody to epitope X or Y could be equally affective in stearically blocking this activa site A. Panal B. A monoclonal antibody binding to the same antigen with equal affinity at site Z, ramote from the active sita A. Because the antibody does not diractly recognize the ective site or any close apitope it fails to neutraliza the antigen. Failure to nautrallze the active site results in a circulating entigan-antibody complex with praserved activity. (Note the difference betwaan the antibodies shown in Penals A and B would not be detectad using e binding assay.)

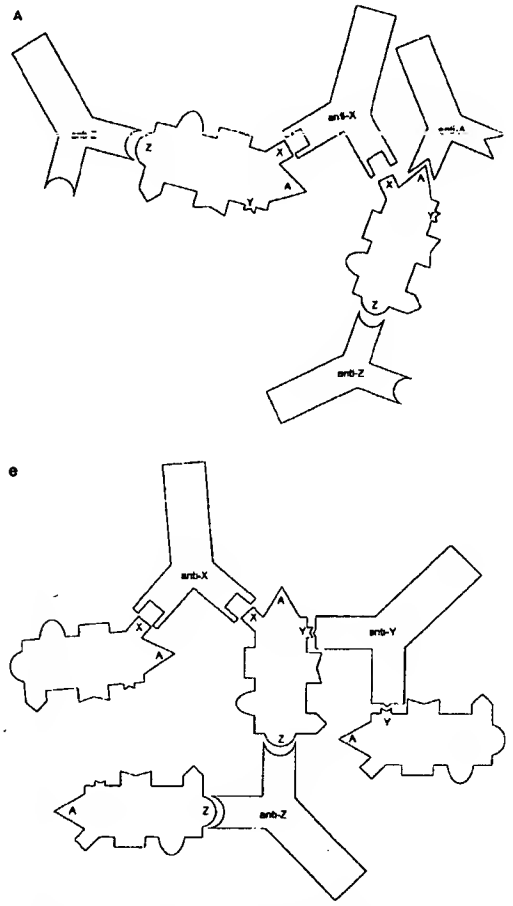

Fig 3. Panel A. Administration of polyclonel entiserum containing antibodies to several epitopes neutralizes the toxin not only by binding the ective site A, but by binding the immediately adjacant epitope X end the distant epitope Z as shown in panel A. This results in the formation of inactive entibody entigen complaxes. Panel B. A potentiel edventaga of a polyclonal antiserum in which even though antibodies to the active site A ere lacking, entibodies to X, Y, end Z produce entigen cross-linking rendering meny of the ective sites of the toxin inaccessible.

nal antiserum containing antibodies to several epitopes neutralizes thc toxin not only by binding the active site A, but by binding the immediately adjacent epitope X and the distant epitope Z as shown in panel A. Panel B shows another potential advantage of a polyclonal antiserum in which even though antibodies to the active site A are lacking, antibodies to X, Y, and Z produce antigen cross-linking, rendering many of the active sites of the toxin inaccessible.

Does this mean that polyclonal antibodies are always more efficacious than their monoclonal counterparts? Certainly not. Should investigators be skilled or lucky enough to select one of the high-affinity monoclonal antibody clones that neutralize the active site on the protcin by binding to it or immediately adjacent to it, a monoclonal antibody could be as cffective or superior to a polyclonal antibody preparation.

Specificity and Antibody Dosing

Balanced against polyclonal antibodies diverse binding ability is the realization that for any total administered dose, a smaller percentage of that dose will be specific for the antigen's active site than would an equivalent amount of a monoclonal antibody directed exclusively against that one epitope. In practice this limitation is overcome by the cooperative binding effects of nonactive site directed antibodies and by the ability to administer a higher total dose of antibody. An example of the efficacy of polyclonal antibodies is seen in some snake antivenoms that may contain as little as 10% specific antibody but are still clinically efficacious.[21] The use of higher antibody doses may only be feasible with polyclonal preparations because of the large disparity in cost between polyclonal and monoclonal antibodies. Although antibodies are usually considered to be very specific molccules, binding to designated effector ccils and to a single antigen, this view is not always correct. For example, IgM class antibodies may nonspecifically bind hydrophobic substances and such nonspecific binding could bc misinterpreted as cross-reactivity.[22,23]

Affinity and Avidity

Although blanket statements cannot be made about antibody-antigen affinity for all monoclonal or polyclonal antibodies, polyclonal preparations often have higher average antigen-antibody affinities. Even though individual antibodies within a polyclonal mixture may have a higher or lower affinity than the mixture's average, the cooperative binding effects of polyclonal antibodies result in overall avidity greater than that of a single monoclonal antibody.[24]

Stability

All antibody preparations if maintained sterile at an appropriate temperature have relatively long shelf-lives. Polyclonal antibodies are

robust, resisting freeze-thaw cycles and enzymatic digestion, and some preparations have long storage potential. For example, the affinity purified polyclonal ovine antidigitalis antibody Digibind (Glaxo Wellcome Inc, Research Triangle Park, NC), has exhibited stable antigen binding properties when stored for up to 4.5 years.[25]

Production Costs

Again, although exceptions certainly exist, polyclonal antisera are usually much less expensive to produce than monoclonal antibodies. It has been estimated that the production cost of a polyclonal antibody is one tenth of that of a monoclonal.[26] In conditions in which repeated therapy may be necessary (eg, sepsis) or where large quantities of antigen must be neutralized (eg, tricyclic antidepressant overdose), the cost of monoclonal therapy may prove prohibitive. For similar reasons it seems unlikely that mixing several monoclonal antibodies to produce a polymonoclonal or multivalent monoclonal will prove economically feasible.

Consistency

A major potential disadvantage of polyclonal antibodies is that, unlike monoclonals, overtime drift can occur in the immunized animals such that the specificity and binding characteristics of the antibody may change. This problem is minimized by using large, stable immunized populations; by obtaining antibodies from animals showing stable production characteristics; and by assuring that the immunogen and immunization schedule are held constant. Quality assurance testing is essential in the production of polyclonal antibodies to assure that binding and neutralization characteristics remain stable over time.

Production Limitations

Another significant limitation of polyclonal antibodies is that not all species are suitable for large-scale antibody production. For example, it is impractical to repeatedly phlebotomize animals below 30 to 50 kg because of the small volumes of blood that can be obtained. In addition, some animals (eg, baboons) are difficult to manage. For these reasons docile farm animals such as horses, goats, pigs, and sheep have been used as major producers rather than rodents, rabbits, dogs, or nonhuman primates.

Adverse Events

Safety of an antibody product can vary greatly depending on the species of origin; type of preparation (whole polyclonal antisera, immunoglobulin fraction, isolated single antibody class, monoclonal antibody, or antibody fragment); antigenic target; binding specificity; and total antibody/protein dose.[2] Because most monoclonal and polyclonal antibodies are ultimately derived at least in part from a nonhuman source, both represent foreign antigen challenges and hence, both are associated with immediate and delayed adverse immunological reactions. Furthermore, because antibody therapy involves the administration of relatively large doses of foreign protein, nonimmunologic flushing reactions are apt to occur when antibodies are rapidly infused. The incidence of early adverse reactions is also influenced by the class of antibody administered. For example, IgM antibodies are excellent at fixing complement, whereas IgG's as a group are less effective. (Some subclasses of IgG are unable to fix complement.) Activation of the complement cascade may be responsible for many non–IgE-mediated reactions.[4]

Allergic and Immunological Reactions

Allergic and immunological reactions to administered antibodies can be classified as classical type I (immediate or anaphylactoid) or type III (immune complex) reactions, or reactions in which antibodies are formed against the foreign proteins but do not produce disease.[15]

In the past, polyclonal antisera from some species have frequently been associated with adverse reactions and these reactions served as a strong impetus for the development of monoclonal antibodies.[4] The adverse reactions to polyclonal antibodies occur largely as a result of administering large amounts of irrelevant contaminating proteins; by exposure to immunoglobulin types not naturally occurring in man; or by reexposure to species specific proteins administered earlier in life.[4] (Many reactions can be traced to reexposure to products obtained from animals commonly used to produce childhood vaccines or antitoxins among which horse serum

is the most common offender.) Exposure to foreign species serum in childhood can result in production of IgE-sensitized mast cells primed for degranulation on repeat exposure to species specific antisera.

Variations in purity and species of origin account for the wide-ranging frequency of adverse reactions when using polyclonal antibodies. For example, some horse-derived snake antivenins contain large amounts of irrelevant protein (sometimes >90%) and the highly immunogenic nonhuman immunoglobulin, IgG.[27] Such equine products can produce immediate allergic reactions in up to 30% of recipients and delayed serum sickness in nearly 70% of treated patients.[5] Antibodies derived from mice, goats, sheep, and humans have substantially lower rates of early adverse reactions, possibly because of the lower likelihood of prior human exposure. For example, use of polyclonal sheep-derived antibody fragments to digitalis have been associated with an observed hypersensitivity reaction rate well below 1%.[28]

From these data one might be tempted to conclude that monoclonal antibodies would have a better side effect profile than nonhuman polyclonal antibodies. However, the incidence of some immunological response to monoclonal antibodies is still substantial and attempts at reducing the formation of antibodies to exogenously administered monoclonal antibodies continue. In addition, because monoclonal antibodies are generated using transformed cells, questions remain about the long-term safety of antibodies potentially containing bacterial or viral genetic material.

Efforts to reduce the antigenicity of monoclonal antibodies has included attempts to produce chimeric and humanized antibodies. Simplistically, chimeric or humanized antibody production is accomplished by combining a murine gene coding for either the variable or hypervariable immunoglobulin region, respectively, with the human gene that codes for the constant regions of the antibody. Chimeric and humanized antibodies have shown a lower rate of antiglobulin formation and longer biological half-lives when compared with monoclonal antibodies from rodent fusion products.[29,30] However, even humanized antibodies exhibit antigenic idiotypic and allotypic sites within the variable regions and host immunological responses are still possible.

Further manipulations are underway to develop small, genetically engineered antibodylike molecules known as immunoadhesins and single-chain antigen-binding proteins. Immunoadhesins are recombinant molecules in which the Fc portion of an antibody molecule is linked to a receptor or epitope binding site of interest. Single-chain antigen-binding molecules are constructed by joining light and heavy chain variable regions by linker peptides resulting in a small molecule lacking the Fc receptor binding domain.[31,32] Absence of an Fc region has the potential advantages discussed earlier in relation to the use of Fab or Fab2 antibodies.[18]

The conclusion that human antibodies are safer than either animal-derived monoclonal or polyclonal antibodies is also erroneous; the risk of infections such as hepatitis, human immunodeficiency virus, and other yet to be discovered agents is small but real. Furthermore, a small minority of recipients with IgA deficiency experience severe immediate reactions when transfused with even trace amounts of human IgA containing material.[4]

Delayed immune reactions are related to the class of antibody selected but seem to be independent of whether the antibody is polyclonal or monoclonal. In general, the longer the exposure to a foreign protein the more likely the host will exhibit an immunological response. High molecular weight antibodies (IgM class) can have very long half-lives and for this reason smaller antibodies (eg, IgG), and even antibody fragments, are likely to be less immunogenic than their larger heavier counterparts. Absence of the Fc portion in digested antibodies or single-chain antigen-binding proteins not only reduces molecular weight but removes the portion of the antibody molecule with potential to bind mononuclear cells Fc receptors.[33]

Although great trepidation exists regarding the host-immune response to exogenously administered antibodies, the clinical significance of such antibody formation is unclear. A large number of native human proteins including transferrin, albumin, and interferon have naturally occurring autoantibodies without clinical evidence of disease. Formation of human anti-mouse (or other species) antibodies (HAMA) is

extremely common in clinical trials but the clinical significance of these antibodies is likely to be small. For example, up to 80% of monoclonal antibody OKT3 recipients develop a HAMA response.[34] Many patients receiving Digibind Fab fragment form antiovine antibodies but no clinical symptoms are associated with this formation.[18] Similarly, nearly 50% of the patients receiving a murine monoclonal antiendotoxin antibody, and nearly 100% of patients receiving one murine monoclonal anti-TNF antibody in clinical trials, have a HAMA response without clinical consequence.[35,36] Furthermore, retreatment with OKT3 or Digibind in the clinical setting has not been associated with a substantial risk of adverse reactions, despite the presence of preformed antidrug antibodies.[34]

Some risks of antibody therapy can be essentially eliminated by appropriate class selection and purification methods. For example, because type I immediate hypersensitivity reactions require the cross-linking of two IgE molecules that recognize prior exposure to a foreign protein, use of an Fab antibody fragment especially one from mice, goats, or sheep greatly reduces this risk by decreasing the chance of cross-linking and by using a species to which previous exposure is unlikely.

The risk of late serum sickness is also dependent to some degree on the class of antibody and prior exposure history. When patients receive an immunoglobulin to which they have no prior exposure it is viewed as immunologically foreign and the host manufactures antibodies to the administered antibody. Host-generated antibodies (predominately IgG) can agglomerate the exogenously administered antibody to form immune complexes, producing a classical pattern of delayed type III reaction or serum sickness.[15,33] The risk of such reactions can be reduced by using small doses of poorly immunogenic antibodies, such as antibody fragments having a short half-life.

THERAPY OF HUMAN SEPSIS

The rapid onset of sepsis precludes use of active immunization (immunity produced by inoculating host with antigen) hence, passive immunization represents the only realistic therapeutic option. Unfortunately, the recent failures of monoclonal antibodies directed against endotoxin, lipopolysaccharide (LPS), and TNF-α have raised questions about the effectiveness of monoclonal antibody therapy for sepsis specifically and has produced doubts about use of antibody therapy in general. Despite recent disappointments, it is clear that both polyclonal and monoclonal antibody preparations have proven efficacy as therapeutic agents in humans.

Several potential targets have been identified for passive antibody therapy in sepsis. These targets are either presumed initiators of the inflammatory septic reaction (eg, Gram-negative bacterial endotoxin, Gram-positive bacterial peptidoglycan) or endogenously produced inflammatory mediators (eg, IL-1, IL-6, TNF-α). The target choice has varied largely dependent on the expertise and prejudice of the investigator, with each target having potential advantages and disadvantages.

Endotoxin Antibodies

Endotoxin (LPS) was the first modern target for monoclonal antibody immunotherapy, chosen because it is a potent inflammatory stimulus; is associated with a poor outcome; and there is a history of at least partial success in treating Gram-negative disease in animals and humans using polyclonal antiserum.[37,38] LPS does not represent an ideal therapeutic target because it is a complex molecule of varying structure, detectable in plasma for only brief periods of time in a minority of patients. Despite the problems associated with LPS as a therapeutic target, two monoclonal antiendotoxin antibodies were developed and underwent large-scale human testing. Both were monoclonal IgM class antibodies, one murine, one chimeric human-murine and were directed against the relatively common core region of LPS of the J5 mutant of *Escherichia coli* 0111:B4.

The first of these drugs, XOMEN E5, (Xoma; Berkeley, CA) has undergone two large clinical trials in human sepsis with disappointing results. Extensive speculation over the failure of this antibody and the similar antiendotoxin, Centoxin-HA1A (Centocor; Malvern, PA), have been published and great emotion surrounds these discussions.[39] Although study design, conduct, or analysis of these trials can be criticized, in the current context it is worth examining data from studies using these antibodies to under-

stand the potential and limitations of monoclonal antibody technology. In vitro, XOMEN E5 has been shown by solid-phase immunoassay to bind to numerous wild type LPS molecules, although the binding is relatively poor requiring large molar excess of antibody.[23,40-43] Unfortunatcly, it is not at all clear that binding translates into biological neutralization of LPS. For example, E5 did not prevent in vitro activation limulus lysate,[44] a commonly used bioassay for endotoxin, or in vitro leukocyte activation or cytokine production, even when the antibody was present in large molar excess.[43,45] Additional studies by Chen,[46] Warren,[47] Wheeler,[48] and others[22] showed that XOMEN E5 resulted in minimal or no protective effect when tested in large and small animal model of endotoxemia in contrast to early reports of protection.[49-51] The key point in these investigations being that endotoxin-antibody binding does not necessarily translate to neutralization.

Initial human dose response testing using XOMEN E5 indicated a serum half-life ranging from 7 to 19 hours and a volume of distribution of 5 to 9 L when given in doses of 0.5 to 15 mg/kg. Phase I studies using doses of 0.1 to 15 mg/kg determined XOMEN E5 to be well tolerated with a half-life of approximately 18 hours.[52] A larger phase II randomized, blinded study tested doses of 2.5 or 7.5 mg/kg administered 24 hours apart. Although no survival difference was observed as a result of treatment, substantial safety information was gained.[53,54] A positive skin test reaction to XOMEN E5 excluded 1/36 patients preliminarily tested. Among those skin tests patients with negative results receiving the drug, adverse events were rare, with one treated patient developing wheezing, and another developing hypotcnsion during drug infusion. Despite the rarity of immediate adverse reactions a sensitive immunoassay detected an IgM or IgG HAMA response to XOMEN E5 in 46% (8/15) of treated patients. HAMA formation was more common among patients receiving the highest doses of XOMEN E5.

In the initial phase III, 468 patient multicenter, randomized blinded trail of XOMEN E5 in sepsis, 30-day mortality was not improved by two doses of XOMEN E5 (2 mg/kg) administered 24 hours apart when examined using an intent to treat analysis.[35] Subgroup analysis detected potential benefit to those with documented Gram-negative sepsis without refractory shock; a discovery that would provide an important hypotheses to be tested in a second clinical trial. Despite the antibody's failure to improve overall survival, significant observations were madc regarding monoclonal antibody use in septic patients. For example, the XOMEN E5 IgM antibody had a substantially shorter half-lifc (11.5 hours) than that of native human IgM. It is not clear if this reduced half-life was the result of rapid clearance because of one of the following: host response to the murine antibody, vascular permeability was increased, or antibody-endotoxin complex was rapidly cleared. XOMEN E5 was found to be safe with an overall hypersensitivity reaction rate of 1.6%, this despite the development of a significant HAMA response in 47% of treated patients treated when tested using a sensitive immunoassay (detection threshold ≤ 1 ng/mL).[35] This reported incidence of HAMA formation may underestimate the true incidence because a substantial proportion of the treated paticnts dicd before having the opportunity to form antibodies. Interestingly, an IgG HAMA response was also detected in 8% of placcbo recipients, suggesting some lack of specificity of the assay. The development of a HAMA response was not associated with any episodes of immune complex disease.

In a second large clinical study to test the hypothesis that septic humans with documented Gram-negative infection would experience benefit from XOMEN E5, no survival advantage could be detected among patients with Gram-negative sepsis in treated versus placebo recipients.[55] XOMEN E5 again had a substantially shorter half life (11.5 hours) than would be expected for endogcnously produced human IgM. This second study confirmed the safety of XOMEN E5 with easily controllable hypersensitivity reactions occurring in only 2.6% of treated patients (1% rash, 1% anaphylactoid, 0.2% local site reaction, 0.5% fever tachycardia). In addition, less than 2% of patients in both the placebo and treated groups had hypotension possibly related to study drug infusion. Despite a 44% incidence of HAMA formation among treated patients compared with 12% in placebo

recipients, no clinically significant episodes of delayed toxicity attributable to HAMA could be detected. The HAMA response followed the expected time course developing in most antibody recipients after day 7.

The second monoclonal antiendotoxin antibody to be clinically tested, Centoxin or HA1A, was a human-murine chimeric antibody. HA1A was reported to bind to numerous endotoxins using ELISA and thin-layer chromatography assays; to protect neutropenic rabbits infected with *Pseudomonas;* and to block the dermal Shwartzman reaction in rabbits.[56-58] Controversy again surrounded the activity of HA1A when studies by Baumgartner,[59,60] Wheeler,[48] Chen,[46] and others[43,61,62] were unable to show neutralization of LPS in various in vivo and in vitro models, and studies in a septic dog model found HA1A use to be associated with a lower survival than placebo.[63] As with XOMEN E5, it is not at all clear that HA1A-endotoxin binding equates to neutralization.

Phase I trials of HA1A using doses of 50 mcg to 100 mg, were conducted in a nonseptic group of 15 patients with cancer[64] and in 34 septic patients to test safety, pharmacokinetics, and immunogenicity.[65] Studies in nonseptic cancer patients indicate a longer half-life of 29 hours than that seen in septic patients but both groups had a similar volume of distribution of 50 mL/kg. The plasma half-life of HA1A measured in septic patients receiving doses of 25, 100, or 250 mg, was approximately 16 hours, similar to that observed in studies using XOMEN E5, and much shorter than that of native IgM antibodies of 5 days.[65,66] The resulting serum half-life produced a plasma level of antibody of 9 mcg/mL at 24 hours. Compared with XOMEN E5, HA1A had a larger volume of distribution 6 L versus 3.8 L and a shorter $t_{1/2}$ of approximately 10 hours versus approximately 16 hours.[52] Importantly, the volume of distribution and half-life were independent of antibody dose or the presence of Gram-negative infection. The half-life of both HA1A and XOMEN E5 are similar to two other monoclonal antibodies tested as anticancer agents.[67] The antibody seemed to be safe with no apparent adverse clinical or laboratory events associated with its administration. Antibodies to HA1A were not detected in a 4- to 21-day period after dosing when tested using a double-antigen radiometric-assay technique.

An initial phase III trial of HA1A in 543 septic humans randomized to receive HA1A or placebo failed to show benefit in an intent to treat analysis. However, subset analysis suggested a survival benefit for patients with Gram-negative bacteremia that persisted among those patients with shock.[68] Unfortunately, the 201 patients with nonbacteremic Gram-negative infections had a trend toward higher mortality and additional analysis failed to show a beneficial hemodynamic effect of HA1A in these patients.[69] Interestingly, among a subset of treated patients it was claimed that HA1A decreased the levels of TNF detectable in plasma early after treatment.[70]

The safety of HA1A was believed to have been confirmed by this study. Adverse events occurred at similar rate in treated and placebo recipients and only one treated patient had a self-limited local hypersensitivity reaction. Testing failed to find a single patient in either the placebo or HA1A group developing an HAMA response. Finally, a nonrandomized, unblinded, postmarketing study of HA1A in France has failed to show a physiological or survival benefit of HA1A.[71] Rather, these uncontrolled data suggest a potentially harmful effect of HA1A in patients without Gram-negative bacterial infection. A second large North American phase III trial of HA1A was undertaken but was terminated when interim analysis of 1,500 patients indicated excess mortality in patients without Gram-negative bacteremia who received HA1A.[72]

In summary, both commercially produced monoclonal antiendotoxin antibodies have produced disappointing results in vivo and in human clinical trials despite the fact that these antibodies clearly bind to a variety of endotoxin molecules in vitro.

Anticytokine Antibodies

Similar temporal limitations exist in using antibodies to cytokine mediators as exist for endotoxin antibodies. Despite the potential limitations of using antibodies to TNF or IL-1, these cytokines represent some of the most potent inflammatory compounds known and the relative constancy of their structure makes them

attractive targets. Pretreatment with polyclonal antibodies, antibody fragments, and monoclonal antibodies to TNF has been clearly shown to prevent LPS and bacteria-induced death and prevent generation of cytokines downstream to TNF (IL-1, IL-6, and IL-8) after intravenous challenge in a wide variety of animal models. Unfortunately, only a narrow therapeutic window exists with posttreatment being significantly less effective than pretreatment in preventing death or morbidity. In addition, when the experimental challenge is administered intraperitoneally, most animal models have not shown benefit of anti-TNF treatment.[73-83] Despite these controversies, several commercially produced anticytokine antibodies are under development.

After showing beneficial effects in primates,[84] Exley,[85] reported preliminary results of a phase I study of a murine monoclonal antibody, CB0006, (Celltech, Slough, UK) to TNF in 14 patients with septic shock. The antibody was free of immediate toxicity and was associated with a rise in mean arterial pressure. Unfortunately, mortality remained high and lack of a control group prevents interpretation of either the physiological or survival data. Using the same TNF antibody in 10 patients, Vincent[86] reported disappointing results, observing minimal hemodynamic and gas exchange improvements in treated patients. Again the lack of a control group precludes interpretation of the data except to say that there does not seem to be major immediate toxicity associated with the antibody. Results of a four-dose level, open-label, phase II study of this antibody showed no impact on survival, despite reducing plasma TNF levels.[36] The 80 treated patients tolerated the infusion well (0.1-10 mg/kg) with no clinical adverse events attributable to the drug and no evidence of hypersensitivity reaction. Overall, a serum half-life of approximately 40 hours was observed. A HAMA response was observed in 49 of 50 patients (98%) but in no case was this associated with a clinical syndrome of immune complex disease.

Results are now available from several studies using the murine monoclonal anti-TNF Bayx 1351 (Miles Inc, Westhaven, CT) including two large human trials in sepsis. Preclinical testing has indicated efficacy several animals models including postchallenge baboon models of Gram-positive and Gram-negative bacteremia, pretreatment models of baboon and monkey endotoxemia, and a pretreatment model of swine challenged with live bacteria.[87-90]

In phase I studies of 20 patients at risk of sepsis given 1 or 2 doses (1-15 mg/kg) of Bayx 1351 and 16 septic patients treated with a single dose of 15 mg/kg, the antibody was well tolerated and had a half-life of approximately 50 hours. Again, although antibody formation to the murine proteins was seen in more than half the patients, there were no discernable adverse results as a result of antibody formation.[91] Nearly 1,500 septic patients were enrolled in the large randomized clinical trials NORASEPT and INTERSEPT. The first of these randomized blinded controlled trials examined 994 patients treated with placebo, 7.5 mg/kg or 15 mg/kg of murine monoclonal IgG_1 anti-TNF. There was no difference in all-cause mortality when comparing treated or placebo recipients at 28 days, despite the presence of a reduced mortality rate at 3 days noted among patients with shock who were given the anti-TNF. The TNF antibody seemed to be well tolerated, with no immediate allergic or hypersensitivity reactions. Short-lived serum sicknesslike reactions were seen in 2.5% of patients receiving the antibody, but resolved within 28 days. Among the TNF antibody-treated patients with serum available for HAMA, testing showed a fourfold or greater rise in titer in 85% of patients by day 28 postinfusion. There were no differences in the number or severity of adverse reactions among patients developing elevated titters of HAMA.[92]

A humanized monoclonal antibody to TNF, CDP 571, has also now been given to 41 patients with sepsis and seems to be well tolerated although limited information is available.[93]

A murine monoclonal Fab_2 fragment directed against human recombinant TNF has now been developed and has undergone limited human testing. This IgG_3 fragment, MAK195F (BASF Knoll Inc; Ludwigshafen, Germany), has an affinity constant of 3.5×10^{-9} M and binds specifically to TNF-α. In vitro, the antibody fragment protects TNF challenged cells in pretreatment and posttreatment studies.[94-97] Because the antibody does not cross-react with TNF of nonhuman species preclinical animal testing has been limited.

Initial uncontrolled human studies of 1 or 3 mg/kg of MAK 195F given repeatedly over 5 days to 20 septic patients showed that the antibody was safe and did not produce a HAMA response. A two-compartment distribution was observed with an initial half-life of 2 to 3 hours, a terminal half life of 20 to 33 hours, and a volume of distribution of 70 to 80 mL/kg. Paradoxically, plasma TNF levels judged by immunoassay increased; however, no biological TNF activity could be detected at baseline or during the study. It is not possible to make conclusions regarding alterations in physiology or survival based on these uncontrolled data.[97]

To date, 122 septic patients treated with nine doses of (0.1, 0.3, or 1 mg/kg) of MAK 195F at 8-hour intervals have been reported. A limited number of patients have received doses as high as 3 mg/kg.[97-99] Like other studies using monoclonal antibodies, the product was well tolerated without evidence of immediate or delayed toxicity. A HAMA response was noted in 40% of antibody recipients. Intent to treat analysis of survival at 28 days has not shown a beneficial effect of antibody treatment. Subset analysis of the treated patients has reportedly detected a treatment benefit for patients with baseline plasma IL-6 levels above 1 ng/mL. Subsequent trials are being organized using IL-6 levels as one inclusion criteria to confirm the hypotheses that patients with substantial IL-6 levels at baseline are those likely to benefit. Although this strategy is scientifically rational, its practicality must be questioned because a rapid standardized bedside test for IL-6 is not clinically available.

Studies in septic humans have been initiated using an ovine polyclonal Fab fragment (CytoTab Therapeutic Antibodies, Inc; Nashville, TN) raised against human TNF. Preclinical studies have shown a survival benefit in a murine model of lethal endotoxemia and beneficial hemodynamic effects in a swine model of endotoxic shock.[100] Testing in humans with the sepsislike Jarisch-Herxheimer reaction caused by louse-borne relapsing fever, have shown a dramatic improvement in fever, tachycardia, and tachypnea caused by the reaction. In addition, profound reductions in plasma TNF, IL6, and IL-8 levels were observed.[101] Additional humans studies are in progress.

SUMMARY

The use of antibody therapy for the treatment of infections and inflammatory disease is well established. Unfortunately, clinical studies of antiendotoxin and anti-TNF monoclonal antibodies have failed to show clear physiological or survival benefit. Little information is available regarding the effect of antibodies to cytokines other than TNF in human sepsis. Limited preclinical data indicate that IL-6 antibodies may abrogate the effects of endotoxin infusion,[102] but no human studies have been performed. Although both monoclonal and polyclonal antibodies have the potential to protect septic humans, at this time it is the polyclonal antibodies that have shown the greatest promise. Each type of antibody possesses specific advantages and limitations, the ultimate effectiveness of which will need to be proven in large randomized clinical trials.

Appendix

Active immunizetion	edministration of e target antigen to e host to induce endogenous production of en entibody response
Adjuvant	a vehicle to deliver antigen and enhance antibody production
Affinity	attractive force of individual antigen-antibody bonds
Antigen	e nonhost molecule capable of inducing en entibody response
Antisere	serum conteining enhanced emounts of entibodies
Avidity	sum of the binding forces of all antigen antibody interections
Chimeric antibodies	see humanized antibody
Cross-linking	formation of antigen antibody lattice complexes
Cooperetive binding	see cross-linking
Cytokine	en endogenously produced protein often inflemmetory in character, eg. TNF, IL 1
Endotoxin	a potent inflammatory integral cell wall component of Gram-negative bacteria
ELISA	an in vitro assay used to detect the level of a compound in a semple by immobilizing it on an antibody coated surfece thet is then imaged by e second entibody linked to en enzyme thet catelyzes the cleevage of an added substrete
Epitope	e structural region on en entigen with unique antibody recognition properties

Appendix (Cont'd)

Feb fragment	e monovalent antibody portion produced by pepein digastlon of a whole antibody evereging 40,000-50,000 MW and composed of the antigan-binding portions of one heavy and one light chain
Fab2 fragment	e divelent antibody portion produced by pepsin digestion of a whole entibody evereging 90,000-100,000 MW and composed of the entigen-binding portions of two heavy and two light chains
Fc fregment	The nonantigen binding tail portion of an antibody molecule composed of the N-terminel regions of both heavy cheins that serves as a cell surface attachment point and complement activator
HAMA	e polyclonel antibody response in humans to the administration of mouse immunoglobulin
Hapten	a small molecule not immunogenic by itself, but capeble of producing a humorel immune response when coupled to e lerger cerrier molecule
Humanized antibody	a nonhuman antibody substantially modified by replecing portions of the heavy chains or heavy and light chains with human proteins in an attempt to reduce antigenicity of the molecule
Hybridoma	the resultent product of fusing an entibody producing plasma cell with an immortelized myelome cell to create e perpetuel clone thet produces e single (monoclonal) antibody
Immortelized myelome cells	transformad myeloma cells capable of forming immortal fusion products with antibody producing B cells resulting in an hybridoma
Immunoadhasin	recombinent molecules in which the Fc portion of an antibody molecule is linked to e receptor or epitope binding site
Immunogen	an antigen deliberetaly administered to en enimal to induce a humoral immune response
Immunoglobulin	collection of antibodies isolated from serum
IL 1	en endogenously producad pleomorphic, proinflammatory cytokine with actions similar to those of TNF
IL 6	a cytokine often observed in septic patients believad possibly to be an integrated marked of the overall severity of inflammetion
Monoclonal antibody	an entibody that recognizes a single epitope on an antigen produced in vitro by fusing a splenocyte to en immortel myeloma cell (hybridoma)
Passive immunization	administretion of antibodies produced in another enimel host in an effort to supplement humoral defenses
Phase I clinicel trial	a human study usually small in scale in which tha sefety end phermecokinetics of a new medication or device is tested in normal subjects or in patients with a disease of interest
Phase II clinical trial	a humen study, usually of 50-200 patients in which safaty of a medication or davice is confirmed and dose ranging is performed
Phese III clinicel triel	a large scale human trial, usually >200 patients in which e medicetion device or procedure is testad to show both safety and efficacy
Plasma cell	e terminally differentieted B-lymphocyte responsible for producing antibodies
Polyclonal antibodies	a mixture of antibodies, produced by immunizing en enimal, which are directed against more than ona epitope
Serum sickness	e systemic illness characterized by fever, nephropathy, end arthritis es e result of endogenously generated antibody production in response to administration of an antibody/immunoglobulin preparation
Single chain antigen binding molecules	antibodylike constructs of light and heavy chain verieble regions by linker peptides resulting in a smell molecule lacking the Fc receptor binding domein
TNF (cechectin)	an endogenously produced, 17.4 kDa pleomorphic inflammatory protein thought to pley en integrel role in the eerly events of inflammation
Type I hypersensitivity reaction	an immediate anaphylactoid reaction resulting from the activation of mast cells by preformed IgE capable of racognizing a foreign antigen
Type III hypersensitivity reection	a delayed reaction resulting from the formation of a human immune response (IgG or IgM) to e foreign entigen

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